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Fatty Acids of Brown and Yellow Fat in Rats

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(Received 10 June 1963)

It is common knowledge that the mammalian body stores fat in its adipose tissue, mainly as triglycerides. This fat can be drawn upon whenever need arises to be utilized as an efficient source of energy. Adipose tissue in a well-nourished animal has a glistening pale-yellow appearance and microscopically the fat is present as one large vacuole within the cell, distending it and pushing the nucleus to one side against the cellular limiting membrane. In some mammals a type of tissue can be identified which also contains large amounts of lipids, again mainly as triglycerides, and which can be found in certain well-defined anatomic locations. This tissue is lobulated, and because of its tanned appearance it has come to be known as brown fat. In contradistinction to the 'usual' yellow adipose tissue, the fat in this case is found as small regular droplets within the cell with the nucleus at or near the centre of the cell. Other morphological differences have been demonstrated by histochemical techniques (Fawcett, 1952) and by electron microscopy (Lever, 1957; Napolitano & Fawcett, 1958). Brown fat is particularly rich in mitochondria and its colour has been shown to be due to its high cytochrome content (Joel & Ball, 1962). Although

it is found most frequently in hibernating mammals, its exact role in the process of hibernation is still obscure. The subject has been reviewed extensively by Johansson (1959).

Although lipids constitute the major component of yellow and brown fat, detailed characterization is lacking except for brown fat of the mouse (Spencer & Dempster, 1962).

The purpose of the present paper is to present analyses of the epididymal fat pad and the interscapular brown-fat pad of albino rats that were maintained on a laboratory chow diet. Results are also included that were obtained on animals deprived acutely of food for comparatively short periods of time.

EXPERIMENTAL

Materials

Animals. Male albino rats weighing 190–290 g. were maintained on pellets of Rockland rat diet for at least 2 weeks before any analysis was performed. They were killed by opening the chest cavity after light ether anaesthesia.

The study was divided into two parts: in the first portion the epididymal fat pad (yellow fat) and the interscapular fat pad (brown fat) were analysed in eight rats that were housed in a group cage and had free access to the food

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pellets and water. In the second part of the study, nineteen rats were housed individually in raised wire-bottom cages to reduce coprophagy, and were starved for 2 days (five rats), 4 days (ten rats) and 6 days (four rats) with free access to water. Ten control animals were handled in a similar manner but were given food *ad libitum*.

Solvents. All solvents were of analytical reagent grade, and, except for diethyl ether, were distilled before use. Light petroleum (Skellysolve F) was treated with conc. sulphuric acid and acidic potassium permanganate before distillation.

Methods

Extraction of total lipids. Total lipids were extracted with chloroform-methanol (2:1, v/v) and purified by the procedure of Folch, Lees & Sloane-Stanley (1957).

Determination of dry weight. The dry weight was calculated by cutting the fat pads into small pieces and drying at 80° overnight.

Determination of lipid phosphorus. Lipid phosphorus was assayed by the method of Zilversmit & Davis (1950).

Determination of cholesterol. Total and free cholesterol were estimated by the procedure of Zak, Dickenman, White, Burnett & Cherney (1954). The amount of cholesterol esters was computed by subtracting the free from the total cholesterol values.

Preparation of methyl esters of fatty acids. The fatty acid composition of the sterol esters, triglycerides and phospholipids of brown and yellow fat was obtained as follows: the tissue was homogenized in a small volume of chloroform-methanol (2:1, v/v) and applied to form a streak on glass plates (20 cm. × 20 cm.) coated with a thin layer (250–275 μ) of Silica Gel G (E. Merck A.-G., Darmstadt, Germany) according to the method of Stahl (Mangold, 1961). Separation was achieved by using as a solvent diethyl ether-light petroleum (b.p. 45–55°)-acetic acid (12:88:1, by vol.). The lipid classes were made visible by exposing the plates to iodine vapour for 30 sec. By running control samples without exposure to iodine vapour, some loss of polyunsaturated fatty acids, especially linoleic acid, was observed in the phospholipid fraction as a result of the exposure to iodine. After being made visible, the zones were scraped off with a brush into tubes fitted with condensers and refluxed with anhydrous methanol-benzene-conc. sulphuric acid (43:5:2, by vol.) for 2 hr. at 80–90° under a slow stream of nitrogen. After being cooled, the solution was diluted with water (1.5 vol.) and extracted five times with light petroleum. The light petroleum washings were combined and dried, the solvent was removed and about 2 ml. of freshly prepared diazomethane in ether solution (Schlenk & Gellerman, 1960) was added to ensure complete esterification. After 15 min. the solution was evaporated and the methyl esters were sublimed for 1 hr. at 60 ± 2° under a vacuum of 0.2 ± 0.15 mm. Hg (Stoffel, Chu & Ahrens, 1959). The esters were washed into test tubes (15 mm. external diam.) with a flared open end and with the other end drawn into microtubes (internal diam. 2–3 mm.). By this procedure, solutions of 50 μ g. or less of methyl esters could be concentrated to very small volumes and easily recovered with a Hamilton micro-syringe for analysis by gas-liquid chromatography.

Preparation of methyl esters of fatty acids from total lipid extracts. The tissues were homogenized in 20 vol. of chloroform-methanol (2:1, v/v) and filtered. The lipids were recovered and saponified, and the non-saponifiable

material was extracted. The free fatty acids were recovered and methylated with boron trifluoride-methanol reagent (Metcalf & Schmitz, 1961).

Gas-liquid chromatography of the methyl esters. 15% (w/w) Butanediol succinate polyester on Gas Chrom Z (Applied Science Laboratories Inc., State College, Pa., U.S.A.) was used as the liquid phase at 187° and individual fatty acid peaks were identified with pure standards obtained from the Hormel Institute.

RESULTS

Lipid composition. Brown fat contains about one-third less extractable lipids than does yellow fat (Table 1). By thin-layer chromatography, triglycerides constitute the major lipid component of both tissues. Larger amounts of free cholesterol are found in brown fat. Differences in the esterified cholesterol values are not significant. Phospholipids as calculated from the lipid phosphorus values comprise about 2% of the total lipids of brown fat and only 0.2% of yellow adipose tissue.

Fatty acid composition of lipid classes in normal rats. No significant differences can be detected between the major fatty acids of the sterol ester fraction of brown and yellow fat, but the triglycerides of brown fat contain more saturated fatty acids, and less monoenoic acids, than do those of yellow fat (Table 2). In the phospholipid fraction of brown fat, linoleic acid constitutes a larger portion of the fatty acids but yellow fat contains more monoenoic acids (Table 2).

Effect of starvation on the fatty acid composition of brown and yellow fat. During starvation there is a gradual loss of total lipids from both tissues (Table 3).

The relative proportion of the fatty acids is altered as the period of starvation is prolonged, and a roughly similar trend appears to be followed in the two tissues (Fig. 1). The most consistent finding is a relative decrease of palmitic acid and to a lesser extent of palmitoleic acid, and an increase in

Table 1. *Lipid classes of brown and yellow fat*

Experimental details are given in the text. Values are given as means ± S.E.M. of results from 12 animals (7 animals for the values for water content).

	Brown fat	Yellow fat
Total lipids (g./100 g. wet wt. of tissue)	59.27 ± 1.41	87.32 ± 0.97
Water content (g./100 g. wet wt. of tissue)	32.21 ± 1.45	9.73 ± 0.49
Free cholesterol (g./100 g. of lipids)	0.17 ± 0.02	0.08 ± 0.02
Cholesterol esters (total minus free cholesterol) (g./100 g. of lipids)	8.02 ± 0.86	6.43 ± 0.36
Phospholipids (mg. of P/100 g. of lipids)	86.08 ± 10.65	8.75 ± 1.15
Phospholipids (mg. of P/100 g. of dry fat-free tissue)	599	259

Table 2. *Fatty acids of major lipid classes of brown and yellow fat*

Experimental details are given in the text. Only the major components that could be identified with the available standard mixtures are listed. Values are given as means $\pm$ s.e.m. of results from seven (sterol esters) or eight (triglycerides and phospholipids) animals. The values underlined differ significantly at the 0.01 level.

Fatty acid (no. of C atoms/ molecule: no. of double bonds/molecule)	Sterol esters		Triglycerides		Phospholipids	
	Brown fat	Yellow fat	Brown fat	Yellow fat	Brown fat	Yellow fat
14:0	5.34 $\pm$ 0.53	4.63 $\pm$ 0.61	3.07 $\pm$ 0.22	1.71 $\pm$ 0.07	1.52 $\pm$ 0.26	2.81 $\pm$ 0.45
16:0	25.93 $\pm$ 1.39	25.68 $\pm$ 1.53	30.40 $\pm$ 1.28	26.66 $\pm$ 0.84	22.81 $\pm$ 1.29	26.37 $\pm$ 1.36
16:1	17.83 $\pm$ 2.30	16.02 $\pm$ 1.68	3.66 $\pm$ 0.31	5.47 $\pm$ 0.21	4.90 $\pm$ 0.83	9.96 $\pm$ 1.23
18:0	6.34 $\pm$ 0.51	6.81 $\pm$ 0.50	6.49 $\pm$ 0.20	2.95 $\pm$ 0.07	16.22 $\pm$ 1.22	12.82 $\pm$ 0.99
18:1	23.21 $\pm$ 1.98	23.98 $\pm$ 2.14	28.41 $\pm$ 0.79	32.39 $\pm$ 0.29	16.62 $\pm$ 1.02	20.99 $\pm$ 0.79
18:2	3.86 $\pm$ 1.62	4.20 $\pm$ 1.22	26.28 $\pm$ 1.14	26.23 $\pm$ 1.51	27.25 $\pm$ 1.90	13.51 $\pm$ 1.89
20:4					7.18 $\pm$ 0.59	6.04 $\pm$ 0.92

Fig. 1. Changes in fatty acid composition of total lipids of (a) brown fat and (b) yellow fat during starvation for: $\square$, 0 days (control); ▨ , 2 days; ▩ , 4 days; $\blacksquare$, 6 days. The vertical lines indicate $\pm$ s.e.m.; * differs from control at the 0.05 level; ** differs from control at the 0.01 level.

Table 3. *Total extractable lipids in starved rats*

Experimental details are given in the text. Values are given as means $\pm$ s.e.m. of results from 35 animals.

Period of starvation (days)	Extractable lipids (% of total)	
	Brown fat	Yellow fat
0	59.27 $\pm$ 1.41	87.32 $\pm$ 0.97
2	34.88 $\pm$ 4.60	79.03 $\pm$ 2.10
4	38.07 $\pm$ 4.57	68.30 $\pm$ 1.59
5	16.88 $\pm$ 4.81	58.56 $\pm$ 9.46
6	10.70 $\pm$ 2.14	44.20 $\pm$ 10.72

oleic acid and linoleic acid. A decrease in myristic acid is also found but only in brown fat whereas an increase in stearic acid is noted in yellow fat.

Histological examination showed that, as the starvation period was prolonged, there was a gradual diminution in size and finally disappearance of the lipid vacuoles from the cells of both brown and yellow fat. At no time, however, did the individual cell of yellow adipose tissue assume the multilocular character of brown fat, i.e. with multiple small lipid vacuoles in the cytoplasm.

DISCUSSION

From previous observations that have been recorded in the literature, it is apparent that brown fat cannot be considered to be identical with yellow adipose tissue: morphological and meta-

bolic differences (Fawcett, 1952; Lever, 1957; Napolitano & Fawcett, 1958; Wertheimer & Shapiro, 1948) have been demonstrated.

Although brown fat in the rat does appear to store fat, mainly as triglycerides, in a manner similar to adipose tissue, the fatty acid composition of the stored lipids is different. This difference can be due to either a selection in the uptake of fatty acids circulating in the serum or a preferential synthesis and/or interconversion from other fatty acids *in situ*.

Brown fat contains more lipid phosphorus, even when this is expressed on a dry fat-free tissue basis. Fawcett (1952) demonstrated that phospholipids occur for the most part in and are intimately associated with mitochondrial structures, and therefore the higher quantities of this lipid in brown fat may correlate well with the greater number of the cellular organelles present in that tissue. Further, the fatty acid pattern of the phospholipid fraction is different from that of yellow fat, containing significantly higher concentrations of linoleic acid.

From the present results, the interscapular brown fat in the rat appears to serve as a storage organ. It tends to react in a manner similar to adipose tissue when the animal is acutely deprived of food. In both tissues there appears to be a relative decrease in palmitic acid as the period of starvation is prolonged. Less consistent changes are also seen with some of the other major fatty acids. Dittmer & Hanahan (1959) have shown that individual fatty acids may be metabolized through different pathways. The differences observed in starved rats indicate either that an attempt is made to conserve the higher-chain fatty acids, which may be true for linoleic acid since it cannot be synthesized to an appreciable extent by the animal, or more likely that some of the shorter-chain fatty acids are mobilized at a faster rate than others as the organism is able to utilize them more efficiently through some preferential metabolic pathway.

SUMMARY

1. Lipids of brown and yellow fat from young male albino rats were analysed by thin-layer and gas-liquid chromatography.

2. The triglyceride fraction of brown fat contained higher quantities of saturated fatty acids but the phospholipids had more linoleic acid.

3. On depriving the animals of food for 2, 4 and 6 days, loss of lipid was observed in both brown and yellow fat, with the suggestion of a greater preferential mobilization of some of the fatty acids, especially palmitic acid.

4. The results suggest that, although brown and yellow fat in the rat appear to fulfil the common function of storage of triglycerides, differences in the fatty acid composition of the phospholipid and triglyceride fractions between the two tissues may indicate some basic differences in the metabolic pathways involved in lipogenesis or lipid mobilization or both.

Acknowledgement is due to Mr T. Robinson for technical assistance. The author is grateful to Mrs Margaret Coultas for statistical analysis of the results and to Mr T. Gallagher for processing the results at the Computer Center of Washington University. This study was supported in part by U.S. Public Health Service Grant no. 2G-897.

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